6,268,342, which is a 371 of PCT/US97/14154, filed Aug. 27, 1997, which is a continuation-in-part of Ser. No. 08/705,790, filed Aug. 30, 1996, now abandoned.

In the claims:

Please substitute the claim set in Appendix A entitled "Clean Version of Pending Claims" for the previously pending claim set. The specific amendment to claim 142 is as follows:

142 (Amended). A method of inhibiting fibrosis in a patient said method comprising administering a therapeutically effective amount of somatostatin or a somatostatin agonist to said patient, provided said fibrosis is not in the kidney, in the lung, in the liver, in the skin, of the central nervous system, in bone or bone marrow, in the cardiovascular system, in an endocrine organ, or in the gastro-intestinal system, and further provided that said fibrosis is not periportal fibrosis.

No new matter is being added by the foregoing amendments.

REMARKS

Reconsideration of the Office Action mailed August 14, 2001, (hereinafter "instant Office Action"), entry of the amendments hereinabove, and withdrawal of the rejection of claims 142 and 143 are respectfully requested. The amendment to claim 142 is made to further prosecution of the present application and is not intended to concede the correctness of the Examiner's position or to prejudice the prosecution of the claims prior to amendment which may be presented in any continuing application of the instant application.

In the instant Office Action, claims 142-143 are listed as pending and claims 142-143 are listed as rejected.

The 35 U.S.C. §101 Rejection

The Examiner has rejected claims 142 and 143 under 35 U.S.C. §101, alleging that claims 142 and 143 claim the same invention

as that of claims 1-29 of prior U.S. Patent No. 6,268,342 (the '342 patent). Applicants respectfully traverse this rejection.

In order to support a double patenting rejection under 35 U.S.C. 101 it must be shown that an accused claim is drawn to *identical* subject matter as a claim of a prior patent. In this regard Applicants respectfully direct the Examiner's attention to MPEP §804, at paragraph II.A., *Statutory Double Patenting - 35 U.S.C. 101*, wherein it is stated:

> In determining whether a statutory basis for a double patenting rejection exists, the question to be asked is: Is the same invention being claimed twice? 35 U.S.C. 101 prevents two patents from issuing on the same invention. *"Same invention" means identical subject matter. Miller v. Eagle Mfg. Co.*, 151 U.S. 186 (1984); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and *In re Ockert*, 245 F.2d 467, 114 USPQ 330 (CCPA 1957).
>
> A reliable test for double patenting under 35 U.S.C. 101 is whether a claim in the application could be *literally* infringed without *literally* infringing a corresponding claim in the patent. *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970). Is there an embodiment of the invention that falls within the scope of one claim, but not the other? *If there is such an embodiment, then identical subject matter is not defined by both claims and statutory double patenting would not exist.* For example, the invention defined by a claim reciting a compound having a "halogen" substituent is not identical to or substantively the same as a claim reciting the same compound except having a "chlorine" substituent in place of the halogen because "halogen" is broader than "chlorine." On the other hand, claims may be differently worded and still define the same invention. Thus, a claim reciting a widget having a length of "36 inches" defines the same invention as a claim reciting the same widget having a length of "3 feet.";

(emphasis added.)

Applying the foregoing it can be seen that the statutory double patenting rejection levied by the Examiner can not stand since the claims of the '342 patent are not identical in scope to claims 42 and 43 of the instant application. Indeed such would be

the case even if claims 42 and 43 were not amended as provided herein. However, notwithstanding the foregoing Applicants have amended claim 142 in order to remove literal overlap between the claimed subject matter of the instant application and that of the '342 patent.

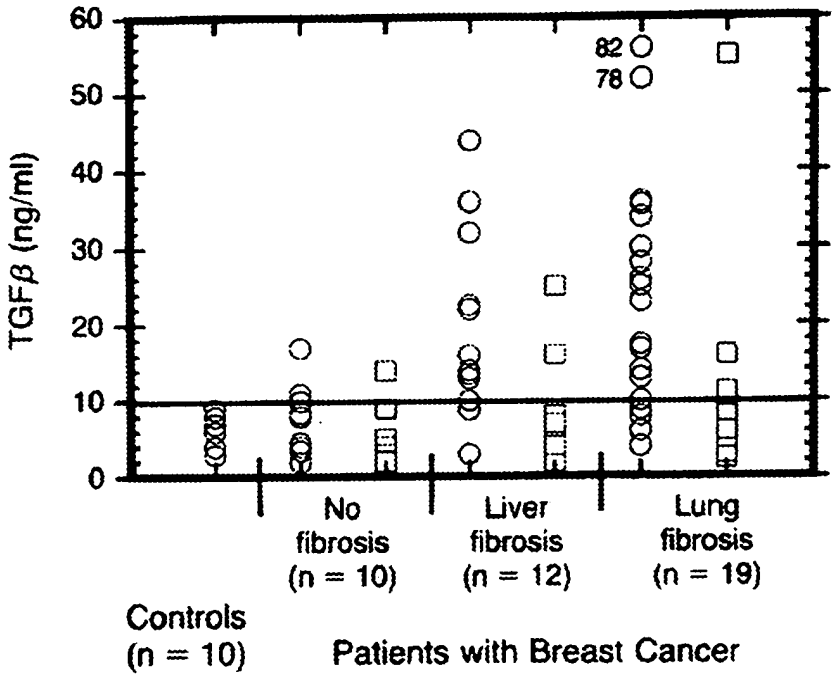
To the extent that claims 1 - 29 of the '342 patent are themselves drawn to various aspects of the invention therein claimed, Applicants' arguments below have been likewise directed to several subsets of logically related claims.

In respect of claims 11 - 16 of the '342 patent Applicants note that said claims are drawn to a method of *inhibiting over-expression of TGF-ß*. In contrast, claims 42 and 43 of the instant application are drawn to a method of *inhibiting fibrosis*. As is well known in the art, inhibition of the over-expression of TGF-ß is not identical to inhibition of fibrosis even though a correlation exists between the incidence of high levels of TGF-ß and incidence of fibrosis.

To be clear in this regard Applicants note that Anscher, M.S., *et al.*, (Transforming Growth Factor ß as a Predictor of Liver and Lung Fibrosis after Autologous Bone Marrow Transplantation for Advanced Breast Cancer, N. Eng. J. Med, 328(22), 1592-98 (1993); hereinafter "Anscher"), teach that:

> pretransplantation TGFß levels were significantly higher in patients in whom hepatic veno-occlusive disease or idiopathic interstitial pneumonitis developed than in the [control population] or the patients without these conditions. The predictive value for the development of either condition was 90 percent or more when pretransplantation plasma TGFß levels were more than 2 SD above the mean established in the controls,

(emphasis added). (See discussion in Anscher, under *Results*). Significantly in this regard are the data Anscher presents in support of the foregoing conclusion, depicted at figure 2 therein, reproduced below:



Reviewing the data it is clear that while elevated TGFß levels are correlated to *increased risk* of fibrosis development, such risk does not reach 100%; i.e., not all individuals with elevated TGFß levels develop fibrosis. Indeed close inspection of figure 2 reveals that, of the 10 individuals in the "No fibrosis" group, at least 3 had TGFß values at or above the 2 SD level discussed in the foregoing passage, with at least one individual exceeding the 2 SD level quite dramatically. (TGFß = approx. 17 ng/ml vs. 2 SD level = 10 ng/ml).

For the Examiner's convenience a copy of Anscher is submitted herewith as Exhibit 1. (The Examiner will note that the copy of Anscher was retrieved from the New England Journal of Medicine web site, and that certain tables and figures were printed separately for the purposes of clarity.)

In light of the foregoing Applicants submit that the literal scope of claims 11 - 16 of the '342 patent is not identical to the literal scope of claims 42 and 43 of the instant application. Accordingly, withdrawal of the rejection of claims 42 and 43 of

the instant application on the grounds of statutory double patenting, to the extent that such rejection is maintained over claims 11 - 16 of the '342 patent, is respectfully requested.

In respect of claims 26 - 29 of the '342 patent Applicants note that the literal scope of said claims encompasses *pharmaceutical compositions*. In contrast, the literal scope of claims 42 and 43 of the instant application encompasses a *method of treatment*, i.e., of inhibiting fibrosis in a patient. Thus the literal scope of claims 26 - 29 of the '342 patent is not identical to the literal scope of claims 42 and 43 of the instant application. Accordingly, withdrawal of the rejection of claims 42 and 43 of the instant application on the grounds of statutory double patenting, to the extent that such rejection is maintained over claims 26 - 29 of the '342 patent, is respectfully requested.

In respect of claim 1 of the '342 patent and the claims that depend directly or indirectly therefrom, (i.e., claims 3 - 8 and 17 - 20), Applicants note that the literal scope of said claims encompasses:

> a method of inhibiting fibrosis in a patient said method comprising administering a therapeutically effective amount of somatastatin [sic, somatostatin] or a somatastatin [sic, somatostatin] agonist to said patient, *wherein said fibrosis is in the kidney, in the lung, in the liver, in the skin, of the central nervous system, in bone or bone marrow, in the cardiovascular system, in an endocrine organ or in the gastro-intestinal system*.

('342 patent, claim 1; emphasis added.). In contrast, the literal scope of claim 42 of the instant application, as presently amended, encompasses:

> [a] method of inhibiting fibrosis in a patient said method comprising administering a therapeutically effective amount of somatostatin or a somatostatin agonist to said patient, *provided said fibrosis is not in the kidney, in the lung, in the liver, in the skin, of the central nervous system, in bone or bone marrow, in the cardiovascular system, in an endocrine organ, or*

> *in the gastro-intestinal system*, and further provided that said fibrosis is not periportal fibrosis.

(emphasis added). Claim 43 depends from claim 42 therefore amendment of claim 42 applies equally to claim 43.

As can be seen, Applicant's have amended claim 42 (and by extension, claim 43) in order to avoid overlap between the literal scope claim 42 and the literal scope of claim 1 (and by extension, claims 3-8 and 17 - 20) of the '342 patent. Accordingly, withdrawal of the rejection of claims 42 and 43 of the instant application on the grounds of statutory double patenting, to the extent that such rejection is maintained over claims 1, 3-8 and 17 - 20 of the '342 patent, is respectfully requested.

In respect of claim 2 of the '342 patent and the claims that depend directly or indirectly therefrom, (i.e., claims 9, 10 and 21 - 25), Applicants note that the literal scope of said claims encompasses:

> a method of inhibiting fibrosis in a patient said method comprising administering a therapeutically effective amount of somatostatin or a somatostatin agonist to said patient, *wherein said fibrosis is induced by chemotherapy, induced by radiation, induced by a drug or a combination of drugs, induced by a disease state, induced by an environmental or an industrial factor, induced by an immune reaction, or induced by a wound.*

('342 patent, claim 1; emphasis added.). In contrast, and as discussed hereinabove, the literal scope of claim 42 of the instant application, as presently amended, encompasses:

> [a] method of inhibiting fibrosis in a patient said method comprising administering a therapeutically effective amount of somatostatin or a somatostatin agonist to said patient, *provided said fibrosis is not in the kidney, in the lung, in the liver, in the skin, of the central nervous system, in bone or bone marrow, in the cardiovascular system, in an endocrine organ, or in the gastro-intestinal system*, and further provided that said fibrosis is not periportal fibrosis.

(emphasis added). Again, claim 43 depends from claim 42 therefore amendment of claim 42 applies equally to claim 43.

Comparing the subject matter of claims 42 and 43 of the instant application to that of claims 2, 9, 10 and 21 - 25 of the '342 patent it can be seen that, whereas the former are concerned with the location of fibrosis to be treated (i.e., what organs or tissues are involved), the latter are concerned with inducements of fibrosis. As such the scope of claims 42 and 43 is not identical with the scope of any of claims 2, 9, 10 and 21 - 25 of the '342 patent. Accordingly, withdrawal of the rejection of claims 42 and 43 of the instant application on the grounds of statutory double patenting, to the extent that such rejection is maintained over claims 2, 9, 10 and 21 - 25 of the '342 patent, is respectfully requested.

The 35 U.S.C. §112 Rejection

The Examiner has rejected claim 143 under 35 U.S.C. §112, second paragraph, alleging that claim 143:

> is substantially duplicative of claim 142. The claim does not further limit or define the antecedent claim

(Instant Office Action, at page 2.). Applicants respectfully direct the Examiner's attention to the text of claim 142, which claims:

> [a] method of inhibiting fibrosis in a patient said method comprising administering a therapeutically effective amount of *somatostatin or a somatostatin agonist* to said patient, ...

In contrast, claim 143 claims:

> [a] method of claim 142, wherein said method comprises administering a therapeutically effective amount of *a somatostatin agonist* to said patient.

Thus contrary to the Examiner's allegation, claim 143 does indeed limit claim 142 since somatostatin has been removed from the genus of compounds to be utilized for treatment. Accordingly, withdrawal of the rejection of claims 43 under 35 U.S.C. §112, second paragraph, is respectfully requested.

Other Matters

Applicants note that an Information Disclosure Statement was filed in the instant application on January 16, 2001. Applicants respectfully request that the Examiner include an initialed copy of Form 1449, submitted therewith, when the Examiner issues the next Office Action.

Applicants also note that claim 42, as amended herein, includes the limitation:

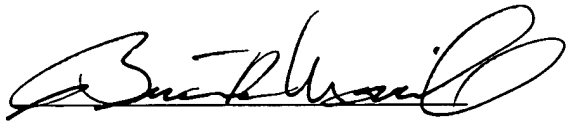
> [...] and further provided that said fibrosis is not periportal fibrosis.

This limitation has been added in light of the disclosure of the Tracy, *et al.* reference (American Journal of Pathology, Vol. 143, No. 6, December 1993) which was made of record in Application No. 09/254,097; i.e., the direct parent application of the instant application. Indeed Tracy *et al.* is among the references cited in the foregoing Information Disclosure Statement.

Based upon the foregoing, Applicants believe that claims 142-143, as amended hereinabove, are in condition for allowance. Prompt and favorable action is earnestly solicited. The Examiner is invited to telephone Applicants' attorney at 508-478-0144 to facilitate prosecution of this application.

Respectfully submitted,

Date: 14-Feb-02



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Serial Number: 09/761,605
Examiner: A. Davenport
Group Art: 1653

Attorney Docket 00537/149003

Exhibit 1

Tissue-Mimicking Gel Phantoms Containing Fibrous Structures for Ultrasound Elastography

[redacted]

[redacted]

The New England Journal of Medicine

ORIGINAL ARTICLE

Volume 328:1592-1598 June 3, 1993 Number 22

Transforming Growth Factor β as a Predictor of Liver and Lung Fibrosis after Autologous Bone Marrow Transplantation for Advanced Breast Cancer

Mitchell S. Anscher, William P. Peters, Herbert Reisenbichler, William P. Petros, and Randy L. Jirtle

ABSTRACT

Background Hepatic veno-occlusive disease and idiopathic interstitial pneumonitis are major causes of morbidity and mortality after bone marrow transplantation. Fibrosis is a characteristic of both conditions, and transforming growth factor β (TGFβ) has been implicated in the pathogenesis of fibrosis.

Methods Using acid-ethanol extraction to remove TGFβ from human plasma and a mink-lung epithelial-cell growth-inhibition assay to measure TGFβ activity, we quantified plasma TGFβ in 10 normal subjects and 41 patients before and after they underwent high-dose chemotherapy and autologous bone marrow transplantation for advanced breast cancer.

Results There was no difference in pretransplantation TGFβ levels between the controls and the patients who did not have hepatic veno-occlusive disease or idiopathic interstitial pneumonitis after transplantation. In contrast, pretransplantation TGFβ levels were significantly higher in patients in whom hepatic veno-occlusive disease or idiopathic interstitial pneumonitis developed than in the controls or the patients without these conditions. The predictive value for the development of either condition was 90 percent or more when pretransplantation plasma TGFβ levels were more than 2 SD above the mean established in the controls.

The New England Journal of Medicine

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ABSTRACT

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Conclusions The plasma TGFβ concentration measured after induction chemotherapy but before high-dose chemotherapy and autologous bone marrow transplantation strongly correlates with the risk of hepatic veno-occlusive disease and idiopathic interstitial pneumonitis after these treatments.

Hepatic veno-occlusive disease is a serious consequence of high-dose chemotherapy or radiotherapy combined with bone marrow transplantation for neoplasia; it occurs in 15 to 50 percent of patients, with a mortality rate of up to 50 percent[1,2,3,4,5,6,7]. The syndrome typically develops one to three weeks after transplantation and is characterized by sudden weight gain, hepatomegaly, ascites, and hyperbilirubinemia[7]; hepatic encephalopathy may also develop.

Similarly, pulmonary complications of bone marrow-transplantation are a major source of morbidity, occurring in 40 to 60 percent of patients[8]. Nonintectious pulmonary complications (idiopathic interstitial pneumonitis) occur in 10 to 25 percent of bone marrow-transplant recipients[8]. This syndrome is characterized by dyspnea, fever, and hypoxemia, with or without diffuse interstitial infiltrates on chest radiography. It occurs 40 to 75 days after transplantation; the mortality rates are high. Both the chemotherapy and the radiotherapy used in the conditioning regimens have been implicated in the development of liver and lung damage after bone marrow transplantation[1,8,9].

Fibrosis is a prominent feature in both the lungs and the liver in patients with these complications[10,11]. Recently, efforts have been directed at elucidating the molecular mechanisms of these fibrotic reactions. Transforming growth factor β (TGFβ) stimulates fibroblasts to migrate to the site of injury, proliferate, and produce collagen; it also inhibits collagen degradation[12]. Thus, it plays an important part in normal wound healing[13,14] as well as in abnormal fibrogenesis. TGFβ has been implicated in the causation of chronic pulmonary fibrosis in rats and mice exposed to bleomycin or cyclophosphamide[15,16,17,18,19,20] and in the development of hepatic fibrosis in rats exposed to radiation[21] or carbon tetrachloride[22,23]. TGFβ may also have a role in fibrotic liver and lung diseases in humans,[24,25,26,27] such as chronic hepatitis,[28] idiopathic pulmonary fibrosis,[29,30] and systemic sclerosis[31,32,33]. Inhibition of TGFβ activity can prevent the development of chronic hepatitis,[28] acute mesangial proliferative glomerulonephritis,[34] and the cirrhotic effects of carbon tetrachloride,[35] providing further evidence for the role of TGFβ in these fibrotic conditions.

Because the level of expression of the gene for TGFβ1 is elevated in both animals and humans with fibrotic liver or lung diseases,[28,30] we postulated that an increase in the release and activation of TGFβ1 in fibrotic tissue would also result in an increase in the circulating level of this growth factor. It may be possible to use the plasma concentration of TGFβ proteins measured before the administration of high-dose chemotherapy to identify patients most prone to the development of lung or liver injury after bone marrow transplantation.

Methods

Patients

Methods

Patients

At the time of this analysis, 102 women with adenocarcinoma of the breast had been treated according to research protocols for bone marrow transplantation at Duke University Medical Center. All patients had either stage IV disease (metastases) or advanced stage II or III disease (more than 10 positive lymph nodes found after axillary dissection) and underwent four cycles of induction chemotherapy followed by high-dose chemotherapy and autologous bone marrow transplantation (Figure 1). The details of this treatment regimen have been previously reported[14]. In brief, the induction regimen consisted of cyclophosphamide, doxorubicin (Adriamycin), and fluorouracil (for stage II or III disease) or doxorubicin, fluorouracil, and methotrexate (for stage IV disease). The high-dose chemotherapy consisted of carmustine, cyclophosphamide, and cisplatin. Radiation therapy was directed at the sites of known metastases (stage IV disease) or to the ipsilateral chest wall, internal mammary nodes, and supraclavicular lymph nodes (stage II or III disease) after autologous bone marrow transplantation.

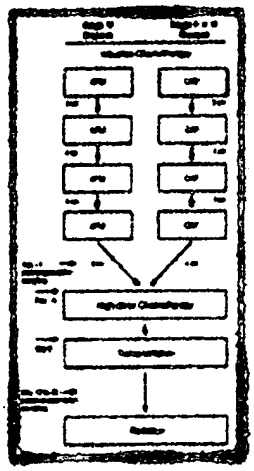

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Figure 1. Treatment Protocol and Plasma Sampling in 102 Women with Breast Cancer.

AFM denotes a regimen of doxorubicin (Adriamycin), fluorouracil, and methotrexate; CAF, a regimen of cyclophosphamide, doxorubicin, and fluorouracil; and high-dose chemotherapy, a regimen of cyclophosphamide, carmustine, and cisplatin. Transplantation of autologous bone marrow was carried out after the high-dose chemotherapy.

Of the 102 patients treated, 12 subsequently had hepatic veno-occlusive disease, 19 had pulmonary fibrosis, and the remaining 71 had neither. Both conditions were defined clinically. Hepatic veno-occlusive disease was indicated by the development of weight gain, hepatomegaly, ascites, and hyperbilirubinemia one to three weeks after transplantation. Pulmonary fibrosis was indicated by dyspnea, fever, and hypoxemia with or without diffuse interstitial infiltrates on chest radiography, beginning 40 to 75 days after transplantation. Other causes of these two syndromes had to be excluded in order to accept these diagnoses. Biopsy was not required. All patients with hepatic veno-occlusive disease or pulmonary fibrosis were included in this analysis. A sample of 10 patients who had neither condition (a sample matching the number of controls, described below) was randomly selected from among all patients enrolled under these protocols whose plasma samples were stored in the archives of the cryopreservation laboratory. Specimens were coded, and TGFβ levels measured, without the investigators' prior knowledge of whether or not the patient had hepatic veno-occlusive disease or pulmonary fibrosis. After the plasma TGFβ levels in the samples were measured, the code was broken and the data were grouped for analysis according to the patients' status for toxic complications (see below).

At the time of this analysis, 102 women with adenocarcinoma of the breast had been treated according to research protocols for bone marrow transplantation at Duke University Medical Center. All patients had either stage IV disease (metastases) or advanced stage II or III disease (more than 10 positive lymph nodes found after axillary dissection) and underwent four cycles of induction chemotherapy followed by high-dose chemotherapy and autologous bone marrow transplantation (**Figure 1**). The details of this treatment regimen have been previously reported[3]. In brief, the induction regimen consisted of cyclophosphamide, doxorubicin (Adriamycin), and fluorouracil (for stage II or III disease) or doxorubicin, fluorouracil, and methotrexate (for stage IV disease). The high-dose chemotherapy consisted of carmustine, cyclophosphamide, and cisplatin. Radiation therapy was directed at the sites of known metastases (stage IV disease) or to the ipsilateral chest wall, internal mammary nodes, and supraclavicular lymph nodes (stage II or III disease) after autologous bone marrow transplantation.

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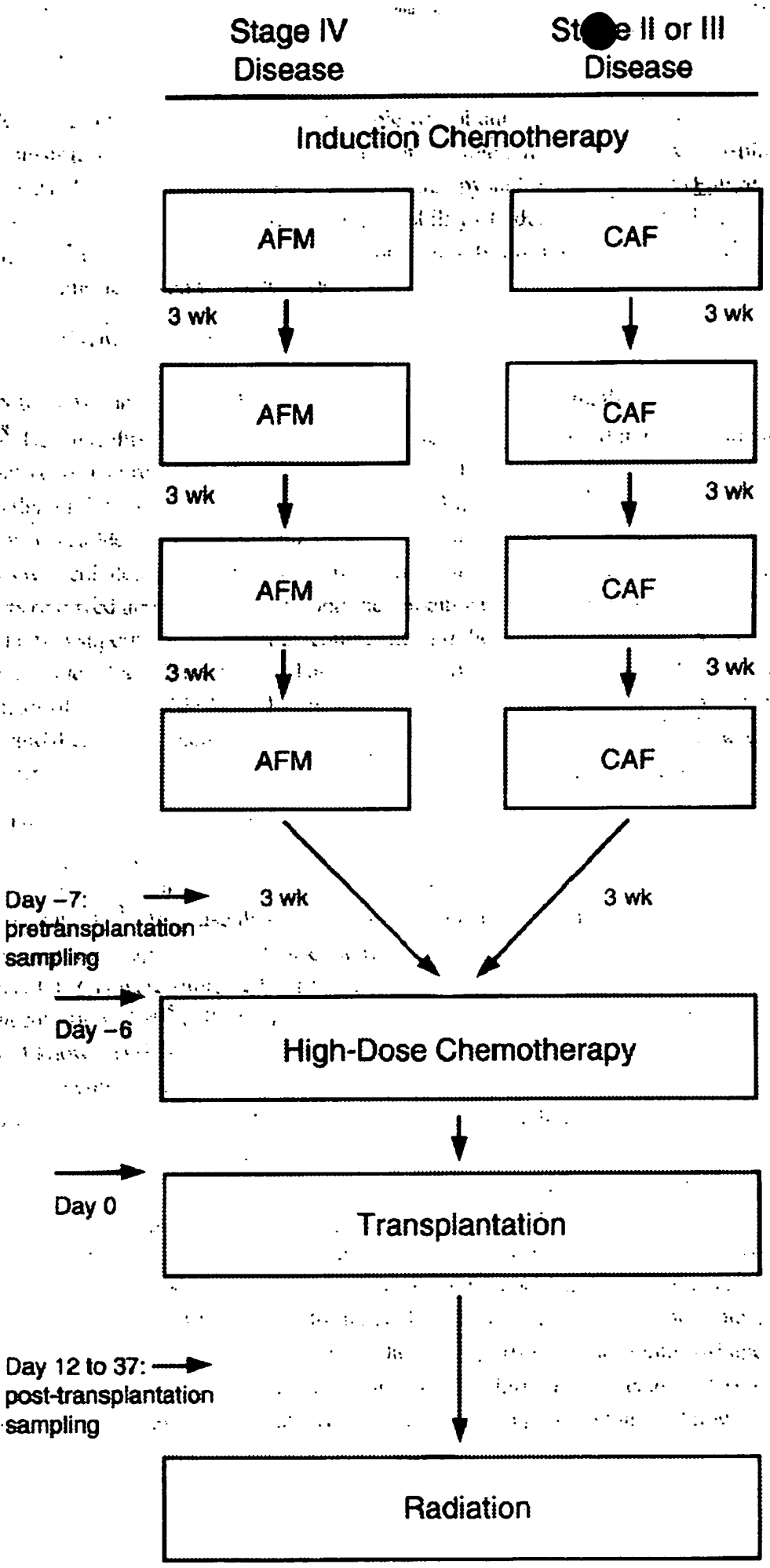

Anscher Fig. 1

Plasma samples were obtained twice. The first sample was obtained after induction chemotherapy but before the administration of high-dose chemotherapy and autologous bone marrow transplantation. The second sample was obtained after the high-dose chemotherapy and transplantation (Figure 1), between 12 and 37 days after the operation, depending on the availability of adequate samples. The findings in the transplant recipients were compared with those in controls -- 10 normal blood donors whose plasma was obtained from the American Red Cross (Charlotte, N.C.).

Extraction of TGFβ from Plasma

Total TGFβ (both active and inactive forms) was isolated from the plasma through acid-ethanol extraction[37,38]. Because this extraction procedure activates TGFβ, we could not determine the amount of active and inactive TGFβ present in the samples. To extract TGFβ, 4 ml of an acid-ethanol solution (375 ml of 95 percent ethanol, 7.5 ml of 12 N hydrochloric acid, 33 mg of phenylmethylsulfonylfluoride, and 1.9 mg of pepstatin) was added to a 1-ml plasma sample previously diluted by a factor of 2 with distilled water. The samples were incubated overnight at 4 °C, then centrifuged at 20,000 x g for 30 minutes at 4 °C. The supernatant was removed and stored at 4 °C, and the remainder of the sample was reextracted and centrifuged. The two supernatants were then combined, and the pH was adjusted to 5.2 to 5.3 with ammonium hydroxide. One milliliter of 2 M ammonium hydroxide was added to 85 ml of supernatant and diluted by a factor of 3 with cold (4 °C) 100 percent ethanol. This solution was incubated at -20 °C for at least two days and then centrifuged. The pellet was resuspended in 6 ml of 1 M acetic acid, dialyzed overnight in 1 percent acetic acid, divided into aliquots, lyophilized, and stored at -20 °C.

Assay for TGFβ

Plasma levels of TGFβ were quantified with the use of an assay measuring the inhibition of the growth of mink-lung epithelial cells[39]. Because this assay is not capable of discriminating among the three isoforms of TGFβ, throughout this paper we simply use the term "TGFβ." In brief, after the MV 1 Lu mink-lung epithelial cells (CCL-64) were subjected to trypsinization and suspended in the assay medium, they were plated at a concentration of 10^5 cells per milliliter. After incubation at 37 °C for 1 hour, TGFβ test samples and standards of known TGFβ1 concentration were added to the wells and incubated at 37 °C for 22 hours. The extent of DNA synthesis was then determined by incubating the cells with ^{3}H-labeled thymidine at 37 °C for an additional four hours. The cells were finally fixed for one hour at room temperature in 1.0 ml of methanol-acetic acid solution (3:1 vol/vol) and washed twice in 80 percent methanol. They were then solubilized in 0.3 N sodium hydroxide, and the radiolabeled DNA was extracted by precipitation with trichloroacetic acid. The amount of radioactivity in the cells exposed to the test samples and TGFβ1 standards was determined with a liquid-scintillation counter. This assay was able to detect amounts of TGFβ ranging from 0.05 to 0.5 ng per milliliter (0.2 to 2 x 10^{-8} nmol per liter), with 50 percent inhibition occurring at a concentration of 0.1 ng per milliliter (0.4 x 10^{-8} mmol per liter). The samples were serially diluted until the quantities of TGFβ present were in the linear portion of the sigmoid-shaped curve for the TGFβ standard. Actual TGFβ levels were then calculated by multiplying the measured TGFβ concentration by the dilution factor. Test samples were always assayed with samples containing known quantities of TGFβ to ensure the reliability of the bioassay.

...determine whether the patient's ... of the test sample was ... TCH_2 ... Plasma samples were obtained twice. The first sample was obtained after induction chemotherapy but before the administration of high-dose chemotherapy and autologous bone marrow transplantation. The second sample was obtained after the high-dose chemotherapy and transplantation (Figure 1), between 12 and 17 days after the operation, depending on the availability of adequate samples. The findings in the transplant recipients were compared with those in controls -- 10 normal blood donors whose plasma was obtained from the American Red Cross (Charlotte, N.C.).

...tatistical Analysis ...

Extraction of TCH_2 from Plasma

...TCH_2 was measured ... control ... patients both before ... after ... administration of ...

Total TCH_2 (both active and inactive forms) was isolated from the plasma through acid-ethanol ... TCH_2. Because ... extraction procedure was not ... TCH_2, we could not determine the amount of ... activated during TCH_2 assays in the samples. To extract TCH_2, 4 ml of plasma acid-ethanol solution (375 ml ... percent ethanol, 2.5 ml of 1.2 N hydrochloric acid, 33 mg of ethylenediaminetetraacetic acid [EDTA], and 1 g of ... was added to a 1-ml plasma sample previously diluted in a ratio of 1:2 with distilled water. The mixture was incubated overnight at 4 °C, then centrifuged at 20,000 x g for 30 minutes at 4 °C. The supernatant was removed and stored at 4 °C, and the remainder of the sample was reextracted and centrifuged. The supernatants were then combined, and the pH was adjusted to 5.2 to 5.5 with the ammonium hydroxide. One milliliter of 2 M ammonium hydroxide was added to 0.85 ml of the supernatant, and the mixture was incubated for 30 minutes at 37 °C. Ammonium sulfate was then added to a concentration of 1 M, and the solution was incubated for 1 hour at 37 °C. The precipitate was collected by centrifugation and then resuspended in 0.5 ml of 1 M acetic acid/sodium acetate buffer, pH 4.5, and dialyzed overnight in 4 percent acetic acid, divided into aliquots, lyophilized, and stored at −20 °C.

Assay for TCH_2

...the patients ...

Plasma levels of TCH_2 were measured with the use of an assay measuring the inhibition of the growth of ... cells. Because this assay is not capable of discriminating among the three isoforms of ... in this paper we simply use the term "TCH_2" in brief, after the MV 1 Lu mink-lung epithelial cells (CCL-64) were subjected to trypsinization and suspended in the assay medium, they were plated at a concentration of 10^5 cells per milliliter. After incubation at 37 °C for 1 hour, TCH_2 test samples or standards of known TCH_2 concentration were added to the wells and incubated at 37 °C for 22 hours. The extent of DNA synthesis was then determined by incubating the cells with ^{3}H-labeled thymidine at 37 °C for an additional four hours. The cells were finally dissolved at room temperature in 1.0 ml of ... methanol-acetic acid solution (9:1 vol/vol) and washed twice with 80 percent methanol. They were then ... chloroacetic acid. The amount of radioactivity collected on the test samples and TCH_2 standards was determined with a liquid-scintillation spectrometer. The assay was sensitive to detection of TCH_2 ranging from 0.05 to 0.5 ng per milliliter (0.6×10^{-8} to 2×10^{-8} mmol per liter) and was precise within 50 percent when occurring at a concentration of 0.1 ng per milliliter (0.4×10^{-8} mmol per liter). The samples were serially diluted until the quantities of TCH_2 present were in the linear portion of the sigmoid shaped curve for the TCH_2 standard. Actual TCH_2 levels were then calculated by multiplying the measured TCH_2 concentration by the dilution factor. Test samples were always assayed with sample containing known quantities of TCH_2 to ensure the reliability of the bioassay.

To determine whether the inhibitory effect of the test samples was due specifically to TGFβ, a neutralizing antibody that recognized TGFβ (R&D Systems, Minneapolis) was added to all the test samples one hour before they were added to the mink-lung cells. Because the antibody was not specific for an individual isoform of TGFβ, we could not determine the relative contributions of the three isoforms to the total plasma concentration. In all test samples the TGFβ antibody was able to neutralize completely the inhibition of 50 percent of the cell growth (data not shown).

Statistical Analysis

Plasma TGFβ was measured in the controls and patients both before and after bone marrow transplantation. Analysis of variance and Scheffe's method of multiple comparisons were used to compare mean values determined before and after transplantation in patients according to whether they subsequently had hepatic veno-occlusive disease, pulmonary fibrosis, or neither condition. Sensitivity, specificity, and predictive values (positive and negative) were calculated on the basis of a cutoff value for plasma TGFβ of 10 ng per milliliter (4×10^{-7} mmol per liter), which was 2 SD above the mean determined in the controls (6 ng per milliliter [2.4×10^{-7} mmol per liter]).

The clinical variables determined in each patient are shown in Table 1. These data were analyzed in the same way as the TGFβ measurements[40]. No clinical information was available for the controls because they were anonymous blood donors. Laboratory values were measured on or as close as possible to the dates on which plasma samples were obtained for measurement of TGFβ (Figure 1), to determine whether there were any differences between the patients in whom hepatic veno-occlusive disease or pulmonary fibrosis developed and the patients without these complications.

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Table 1. Clinical Characteristics Evaluated in 102 Women with Breast Cancer

Results

The characteristics of the 41 patients who underwent autologous bone marrow transplantation for advanced breast cancer are shown in Table 2. The patients in whom pulmonary fibrosis or hepatic veno-occlusive disease later developed and the patients without these complications were similar in all respects except that the group with hepatic veno-occlusive disease included patients with distant metastases who had received chemotherapy or radiotherapy before they were enrolled in the transplantation program. The mortality rates for pulmonary fibrosis and hepatic veno-occlusive disease were 26 percent and 17 percent, respectively (Table 2).

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View this table: **Table 1.** Clinical Characteristics Evaluated in 102 Women with Breast Cancer.
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Results

The characteristics of the 41 patients who underwent autologous bone marrow transplantation for advanced breast cancer are shown in Table 2. The patients in whom pulmonary fibrosis or hepatic veno-occlusive disease later developed and the patients without these complications were similar in all respects except that the group with hepatic veno-occlusive disease included patients with distant metastases who had received chemotherapy or radiotherapy before they were enrolled in the transplant program. The mortality rates for pulmonary fibrosis and hepatic veno-occlusive disease were 25 percent and 17 percent, respectively (Table 2).

Hematologic factors†
- White-cell count
- Hemoglobin
- Hematocrit
- Platelet count
- Prothrombin time
- Activated partial-thromboplastin time

Biochemical factors†
- Uric acid
- Sodium
- Potassium
- Chloride
- Bicarbonate
- Blood urea nitrogen
- Creatinine
- Calcium
- Magnesium
- Phosphorus
- Albumin
- Alkaline phosphatase
- Aspartate aminotransferase
- Alanine aminotransferase
- Lactate dehydrogenase
- Total bilirubin
- Direct bilirubin

Pulmonary function (measured before induction chemotherapy or transplantation)
- Forced vital capacity
- Forced expiratory volume in one second
- Vital capacity
- Total lung capacity
- Carbon monoxide diffusion capacity
- Expiratory reserve volume
- Functional residual capacity

Treatment factors
- Previous chemotherapy before enrollment for transplantation
- Previous radiation therapy before enrollment for transplantation
- Use of peripheral-blood-cell progenitors during transplantation
- Duration of carmustine infusion during transplantation
- Use of colony-stimulating factor during transplantation

Tumor factors
- Maximal tumor size at diagnosis
- Number of positive nodes at diagnosis

Pharmacokinetics (high-dose chemotherapy only)
- Area under the concentration–time curve for carmustine and cyclophosphamide (data not available for cisplatin)

---

*There were no significant differences in the mean values for these clinical factors between the group of patients who did not have toxic complications and the groups that did ($P>0.1$ in all cases).

†Hematologic and biochemical factors were measured on or as close as possible to the dates on which plasma samples were obtained for measurement of TGFβ.

View this table: [in this window] [in a new window]

Table 2. Characteristics of Patients Undergoing Autologous Bone Marrow Transplantation for Breast Cancer.

The TGFβ concentrations in each patient and control are shown in Figure 2; the solid line at 10 ng per milliliter represents the TGFβ level 2 SD above the mean value of 6.1 ng per milliliter (2.4×10^{-7} nmol per liter) determined in the controls (10 healthy blood donors). The mean TGFβ concentrations in each study group are shown in Figure 3. When we compared the TGFβ levels measured in the patients before transplantation with the levels in the controls, we found no significant difference ($P>0.1$) between the controls and the patients who did not have hepatic veno-occlusive disease or pulmonary fibrosis after transplantation. In contrast, the pretransplantation TGFβ levels in patients who later had hepatic veno-occlusive disease or pulmonary fibrosis were significantly higher ($P = 0.003$) than those in the controls and the patients without fibrotic changes in their lungs or liver.

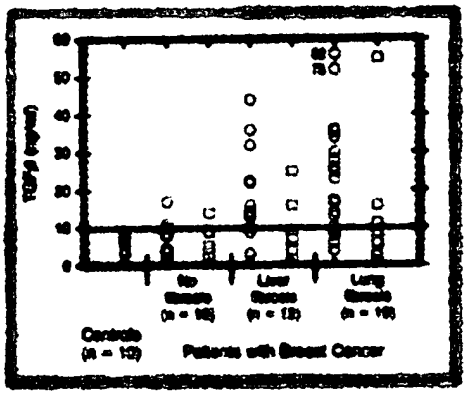

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Figure 2. Individual TGFβ Plasma Concentrations in the Four Study Groups.

Healthy blood donors served as controls. One group of patients did not have pulmonary fibrosis or hepatic veno-occlusive disease after high-dose chemotherapy and autologous bone marrow transplantation (No fibrosis); the other group had clinical hepatic veno-occlusive disease (Liver fibrosis) or pulmonary fibrosis (Lung fibrosis).

TGFβ was measured before (circles) and after (squares) high-dose chemotherapy and transplantation (Figure 1, shown as time of the regimen). The solid horizontal line indicates the value (10 ng per milliliter, or 2 SD above the mean value determined in the controls) that was used as a cut-off point to determine the ability of pretransplantation TGFβ concentrations to predict the development of hepatic or pulmonary fibrosis after transplantation. To convert values for TGFβ to millimoles per liter, multiply by 4×10^{-8}.

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Table 2. Characteristics of Patients Undergoing Autologous Bone Marrow Transplantation for Breast Cancer.

The TOFP concentrations in each patient and control are shown in Figure 2: the solid line at 10 ng per milliliter represents the TOFP level 2 SD above the mean value of 6.1 ng per milliliter (2.4×10^{-7} mmol per liter) determined in the controls (10 healthy blood donors). The mean TOFP concentrations in each study group are shown in Figure 3. When we compared the TOFP levels measured in the patients before transplantation with the levels in the controls, we found no significant difference ($P>0.1$) between the controls and the patients who did not have hepatic veno-occlusive disease or pulmonary fibrosis after transplantation. In contrast, the pretransplantation TOFP levels in patients who later had hepatic veno-occlusive disease or pulmonary fibrosis were significantly higher ($P = 0.003$) than those in the controls and the patients without fibrotic changes in their lungs or liver.

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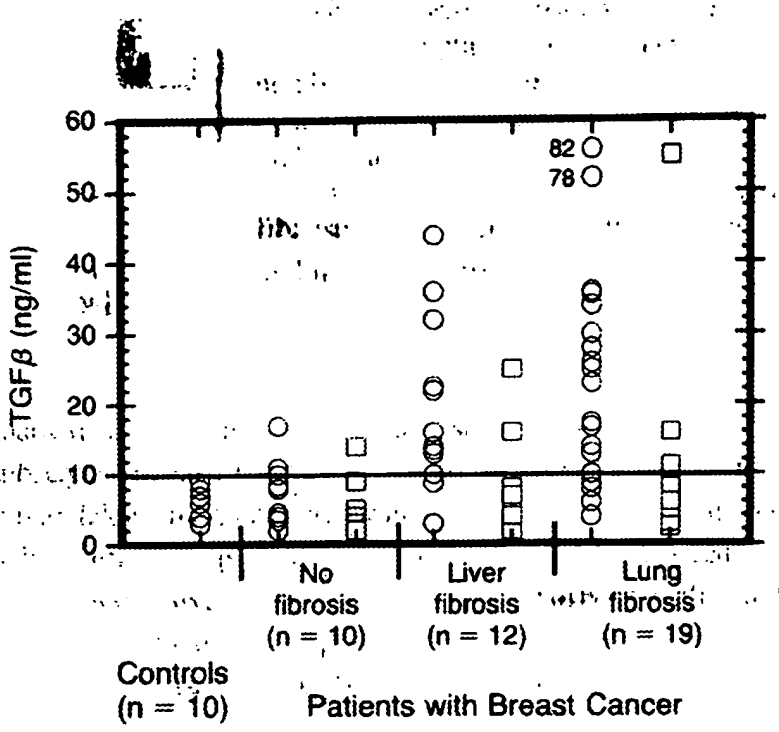
Figure 2. Individual TOFP Plasma Concentrations in the Four Study Groups.

Healthy blood donors served as controls. One group of patients did not have pulmonary fibrosis or hepatic veno-occlusive disease after high-dose chemotherapy and autologous bone marrow transplantation (No fibrosis); the two other groups had either hepatic veno-occlusive disease (Liver fibrosis) or pulmonary fibrosis (Lung fibrosis).

TOFP was measured before (circles) and after (squares) high-dose chemotherapy and transplantation (Figure 1 shows the timing of the regimens). The solid horizontal line indicates the value (10 ng per milliliter, or 2 SD above the mean value determined in the controls) that was used as a cutoff point to determine the ability of pretransplantation TOFP measurement to predict the development of hepatic or pulmonary toxicity after transplantation. To convert values for TOFP to millimoles per liter, multiply by 4×10^{-8}.

Variable	Patients without Fibrosis (N = 10)	Patients with Fibrosis	
		Liver (N = 12)	Lungs (N = 19)
Age (yr)			
Mean	41	40	39
Range	32–53	32–46	30–47
Tumor size (cm)*			
Mean	4.2	4.2	3.4
Range	1.2–12	2–11	1–10
No. of positive nodes			
Mean	15	12	14
Range	10–39	0–33	10–33
Patients given chemotherapy before enrollment for transplantation (%)	0	5 (42)	0
Patients given previous radiation therapy (%)	0	2 (17)	0
Patients with distant metastases (%)			
No metastases	10	6 (50)	19 (100)
Lung	0	0	0
Liver	0	2 (17)	0
Other site	0	4 (33)	0
Patients dying of toxic complications (%)	—	2 (17)	5 (26)

*Measured before induction chemotherapy.



Anscher Fig. 2

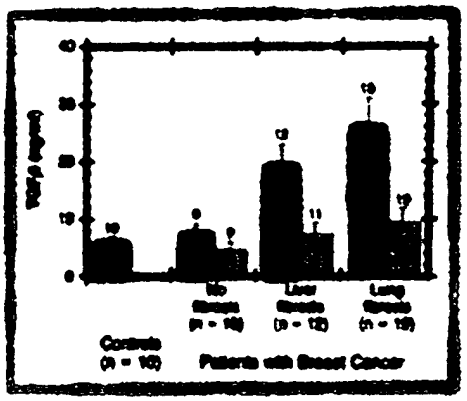


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Figure 2. Mean (±SE) TGFβ Concentrations in the Controls and Patients.

The legend to Figure 2 describes the four study groups. TGFβ was measured before (solid bars) and after (dotted bars) bone marrow transplantation (Fig. 1 shows the times of plasma sampling). The numbers above the bars are the numbers of subjects in each group in whom TGFβ was measured. The patients with fibrosis had significantly higher plasma TGFβ levels before transplantation ($P = 0.003$) than either the controls or the patients without fibrosis. To convert values for TGFβ to milligrams per liter, multiply by 4×10^{-3}.

After autologous bone marrow transplantation, there was a significant decrease ($P<0.001$) in the TGFβ levels of patients with subsequent hepatic veno-occlusive disease or pulmonary fibrosis (Figure 2 and Figure 3). This decrease paralleled a marked decrease in the platelet count after the high-dose chemotherapy (Table 3). In contrast, the plasma TGFβ levels remained unchanged ($P>0.1$) in the patients who did not have hepatic veno-occlusive disease or pulmonary fibrosis, even though their platelet counts decreased to the same extent as the counts of the patients who did have these complications. Although there was a significant difference in pretransplantation TGFβ levels between the groups with hepatic veno-occlusive disease and pulmonary fibrosis and the group without these developments, as noted above, there was no significant ($P>0.1$) difference in the post-transplantation levels among these three groups.

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Table 3. Mean (±SEM) TGFβ Levels and Platelet Counts before and after Autologous Bone Marrow Transplantation.

To test the usefulness of the TGFβ level as an indicator of an increased risk of hepatic veno-occlusive disease or pulmonary fibrosis after high-dose chemotherapy and autologous bone marrow transplantation, the sensitivity, specificity, and the positive and negative predictive values of this marker were calculated. To make these calculations, the upper limit of normal TGFβ levels in plasma was set at 10 ng per milliliter, which was 2 SD above the mean value in normal subjects (Figure 2). The resulting values (Table 4) showed that the TGFβ level measured in plasma after induction chemotherapy but before high-dose chemotherapy and transplantation was a very good indicator of which patients would subsequently have pulmonary fibrosis or hepatic veno-occlusive disease (or both) after chemotherapy and transplantation. If the plasma concentration of TGFβ was greater than 10 ng per milliliter, it was possible to predict with more than 90 percent accuracy that either hepatic veno-occlusive disease or pulmonary fibrosis would develop (i.e., the positive predictive value was >90 percent).

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Figure 3. Mean (±SEM) TGFβ Concentrations in the Controls and Patients.

The legend to Figure 2 describes the four study groups. TGFβ was measured before (solid bars) and after (hatched bars) bone marrow transplantation (Fig. 1 shows the times of plasma sampling). The numbers above the bars are the numbers of subjects in each group in whom TGFβ was measured. The patients with [redacted] had significantly higher plasma TGFβ levels before transplantation ($P = 0.003$) than either the controls or the patients without [redacted]. To convert values for TGFβ to millimoles per liter, multiply by 4×10^{-8}.

After autologous bone marrow transplantation, there was a significant decrease ($P<0.001$) in the TGFβ levels of patients with subsequent hepatic veno-occlusive disease or pulmonary [redacted] (Figure 2 and Figure 3). This decrease paralleled a marked decrease in the platelet count after the high-dose chemotherapy (Table 3). In contrast, the plasma TGFβ levels remained unchanged ($P>0.1$) in the patients who did not have hepatic veno-occlusive disease or pulmonary [redacted], even though their platelet counts decreased to the same extent as the counts of the patients who did have these complications. Although there was a significant difference in preparative-plasma TGFβ levels between the groups with hepatic veno-occlusive disease and pulmonary [redacted] and the group without these developments, as noted above, there was no significant ($P>0.1$) difference in the post-transplantation levels among these three groups.

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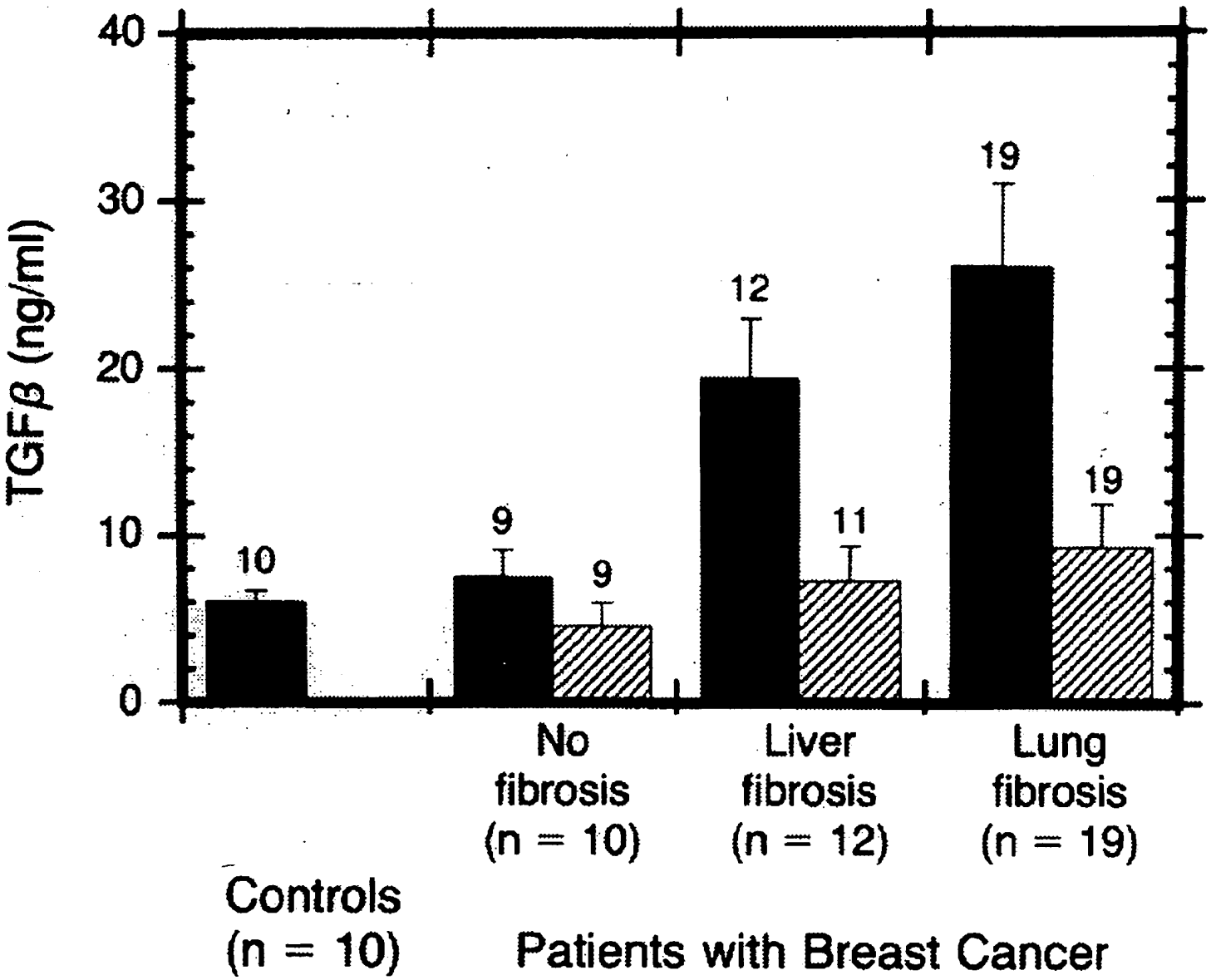

Anscher Fig. 3

Variable	Patients without Fibrosis	Patients with Fibrosis				
		Liver			Lungs	
	Before	After	Before	After	Before	After
TGFβ level (ng/ml)*	7.6±1.6	4.6±1.4	19.5±3.5	7.3±2.1	26.1±4.9	9.3±2.6
Platelet count ($\times 10^{-3}$/ml)	172±23	28±6.7	172±18	25±8.4	212±14	29±3.6

*To convert values to millimoles per liter, multiply by 4×10^{-8}.

Anscher Table 3

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Author Block Redacted

Abstract Redacted

Keywords Redacted

View this table: [in this window] [in a new window] Table 4. Pretransplantation Measurement of Plasma TGFβ as a Predictor of Liver and Lung Fibrosis after High-Dose Chemotherapy and Autologous Bone Marrow Transplantation.

We also attempted to determine whether the development of pulmonary fibrosis or hepatic veno-occlusive disease was associated with any of the variables listed in Table 1. We could find no significant difference in the mean values for these clinical factors between the patients with these complications after transplantation and those without them ($P>0.1$ in all cases). Furthermore, there was no correlation between any of the factors and the pretransplantation TGFβ levels in the three groups of patients (data not shown).

To explore the possibility that TGFβ might be produced by the tumor, the relation between tumor burden, as measured by the maximal tumor dimension and the number of lymph nodes involved by cancer, and the plasma TGFβ concentration before transplantation was determined (Table 5). There were no significant differences in pretransplantation TGFβ levels when the patients were compared according to the number of involved lymph nodes or the greatest measurable tumor dimension (before induction chemotherapy).

View this table: [in this window] [in a new window] Table 5. TGF(β) Concentration in Relation to Tumor Burden in Patients with Liver or Lung Fibrosis.

We also considered the possibility that patients with stage IV cancer who had previously received chemotherapy (before their enrollment in the bone marrow transplantation study) might be at increased risk for toxic complications, as compared with patients who had not previously received chemotherapy. This comparison could be made only in the group with hepatic veno-occlusive disease, since no patient in the other groups had been previously treated with chemotherapy. We found no difference ($P>0.1$) in the pretransplantation TGFβ levels between the patients who had received previous chemotherapy and those who had not, which suggested that previous chemotherapy did not necessarily increase the risk of hepatic veno-occlusive disease in this group.

Discussion

Hepatic veno-occlusive disease and pulmonary fibrosis are major causes of morbidity and mortality after bone marrow transplantation for cancer. Many clinical factors define patient populations at increased risk for the development of these complications,[1,2,3,4,5,6] but none of these clinical factors have been useful in assessing the risk in an individual patient. Our study indicates that the plasma TGFβ concentration, if measured after induction chemotherapy, strongly correlates with the development of pulmonary fibrosis or hepatic veno-occlusive disease after high-dose chemotherapy and autologous bone marrow transplantation.

Patients most prone to pulmonary fibrosis or hepatic veno-occlusive disease after high-dose chemotherapy and autologous bone marrow transplantation have elevated TGFβ levels before transplantation. A positive

Patients must prove to pulmonary [illegible] or hepatic veno-occlusive disease after high-dose chemotherapy and autologous bone marrow transplantation have elevated TGF-β levels before transplantation. A positive

bone marrow transplantation

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Hepatic veno-occlusive disease and pulmonary [illegible] are major causes of morbidity and mortality after

Discussion

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Table 4. TGF-β Concentration in Relation to Tumor Burden in Patients with Liver or Lung Fibrosis.

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any of the factors and the pretransplantation TGF-β levels in the three groups of patients (data not shown).

transplantation and those without them ($P>0.1$ in all cases). Furthermore, there was no correlation between in the mean values for these clinical factors between the patients with these complications after disease was associated with any of the variables listed in Table 1. We could find no significant difference

We also attempted to determine whether the development of pulmonary fibrosis or hepatic veno-occlusive

Liver and Lung Fibrosis after High-Dose Chemotherapy and Autologous Bone Marrow Transplantation

View this table: **Table 6.** Prognostic Evaluation Measurement of Plasma TGF-β as a Predictor of

Variable	Liver Fibrosis	Lung Fibrosis
	percent	
Sensitivity	75	74
Specificity	89	89
Positive predictive value	90	93
Negative predictive value	73	62

Anscher Table 4

Tumor-Burden Factor	TGFβ (ng/ml)	P Value
No. of positive nodes*		
≤12	22.8	>0.1
>12	17.9	
Tumor size (cm)†		
≤3.5	19.4	>0.1
>3.5	20.9	

*Mean number of positive nodes, 12.

†Mean tumor size, 3.5 cm.

Anscher Table 5

test for TGFβ has a positive predictive value of more than 90 percent for the development of either hepatic veno-occlusive disease or pulmonary fibrosis in a given patient. It may be possible to use TGFβ plasma levels to individualize therapy and thus reduce the risk of both complications.

We chose the assay system we used in this study because of its ability to detect very low levels of TGFβ. Although enzyme-linked immunosorbent assays for the quantification of TGFβ[47] are less sensitive than our biologic assay, our results demonstrate that enzyme-linked immunosorbent assays should have sufficient sensitivity to permit rapid screening for patients most prone to fibrotic changes (i.e., patients with plasma levels of TGFβ1 above 10 ng per milliliter [4×10^{-7} mmol per liter]).

The cause of elevated plasma levels of TGFβ in patients who ultimately have hepatic or pulmonary fibrosis is not known. Platelets are the principal source of TGFβ in humans, but an artifactual disruption of platelets seems unlikely. For TGFβ levels to become falsely elevated in the patients we studied, blood samples would have had to have been obtained shortly after platelet destruction occurred, since the half-life of TGFβ in the blood is only a few minutes. Also, the putative destruction of platelets by drugs or venipuncture would have had to have occurred only in the patients who ultimately had fibrosis. Finally, all patients treated with high-dose chemotherapy and autologous bone marrow transplantation had a decrease in TGFβ concurrent with chemotherapy-induced thrombocytopenia.

The elevation of plasma levels of TGFβ in patients with hepatic veno-occlusive disease or pulmonary fibrosis also does not appear to be related to their tumor burden. Some factor other than the tumor is apparently responsible for the elevated TGFβ levels in patients with these complications.

Increased synthesis or activation of TGFβ or decreased degradation of this growth factor (or some combination of these processes) is a possible response to induction chemotherapy in patients who subsequently have hepatic veno-occlusive disease or pulmonary fibrosis. Hoyt and Lazo[20] have shown that strain-specific variations in TGFβ messenger RNA in the lungs of mice correlate with differences in susceptibility to cyclophosphamide-induced pulmonary fibrosis. Genetic differences may also occur in human responses to chemotherapeutic agents.

TGFβ is normally secreted from cells as a glycosylated latent complex that contains phosphorylated mannose residues[48]. It must be dissociated from this complex to become biologically active. The latent complex of TGFβ1 binds to the receptor that accepts both glycoproteins containing mannose-6-phosphate and insulin-like growth factor II,[49] and this binding has been shown to facilitate the activation of the TGFβ1 molecule by proteolytic enzymes[19]. It is possible that this activation process is augmented in patients in whom hepatic veno-occlusive disease or pulmonary fibrosis develops, and consequently more mature TGFβ would be present in the plasma. We have observed an increased concentration of TGFβ in hepatocytes with increased numbers of mannose-6-phosphate–insulin-like growth factor II receptors when the liver is undergoing regeneration[50] or has been exposed to the liver-tumor promoter phenobarbital[51]. Whether a concomitant increase in the level of TGFβ1 and the number of mannose-6-phosphate–insulin-like growth factor II receptors also occurs in the liver and lungs of humans after exposure to chemotherapeutic agents, radiation, or other insults resulting in fibrosis is unknown.

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levels in individuals receiving therapy and thus reduce the risk of both complications.

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References

1. Nesbitt JC, Grosshans KA, Hilgers RD, Steinkamp JA. Fluorescence flow cytometric characterization of cryptosporidia oocysts. Appl Environ Microbiol 1992;58:2047-54.
2. Current WL, Bryan RT, Gyorkos TW, et al. Comparison of the cryptosporidiosis in immunocompetent and immunocompromised persons. Am J Trop Med Hyg 1986;35:487-96.
3. Soave R, Armstrong D. Cryptosporidium and cryptosporidiosis. Rev Infect Dis 1986;8:1012-23.
4. Navin TR, Juranek DD, Preblud SR. Cryptosporidiosis: clinical, epidemiologic, and parasitologic review. Rev Infect Dis 1981;3:1-22.
5. Current WL, Reese NC, Ernst JV, et al. Human cryptosporidiosis in immunocompetent and immunodeficient persons. N Engl J Med 1983;308:1252-7.
6. Meisel JL, Perera DR, Meligro C, Rubin CE. Overwhelming watery diarrhea associated with a Cryptosporidium in an immunosuppressed patient. Gastroenterology 1976;70:1156-60.
7. Hoxie NJ, Davis JP, Vergeront JM, Nashold RD, Blair KA. Cryptosporidiosis-associated mortality following a massive waterborne outbreak in Milwaukee, Wisconsin. Am J Public Health 1997;87:2032-5.
8. Casemore DP, Wright SE, Coop RL. Cryptosporidia--human and animal epidemiology. In: Reduker DW, Speer CA, eds. Parasitic protozoa. Vol. 3. San Diego: Academic Press, 1997:65-92.
9. Navin TR, Weber R, Vugia DJ, et al. Declining outbreaks of cryptosporidiosis in New York State, 1992-1996. Am J Epidemiol 1999;150:1264-9.
10. Hoxie NJ, Davis JP, Vergeront JM, Nashold RD, Blair KA. Cryptosporidiosis-associated mortality following a massive waterborne outbreak in Milwaukee, Wisconsin. Am J Public Health 1997;87:2032-5.
11. Casemore DP, Wright SE, Coop RL. Cryptosporidia--human and animal epidemiology. In: Reduker DW, Speer CA, eds. Parasitic protozoa. Vol. 3. San Diego: Academic Press, 1997:65-92.
12. Navin TR, Weber R, Vugia DJ, et al. Declining outbreaks of cryptosporidiosis in New York State, 1992-1996. Am J Epidemiol 1999;150:1264-9.

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References

1. McDonald GB, Shulman HM, Wolford JL, Spencer GD. Liver disease after human marrow transplantation. Semin Liver Dis 1987;7:210-229.[Medline]
2. McDonald GB, Sharma P, Matthews DE, Shulman HM, Thomas ED. Venocclusive disease of the liver after bone marrow transplantation: diagnosis, incidence, and predisposing factors. Hepatology 1984;4:116-122.[Abstract]
3. McDonald GB, Shulman HM, Sullivan KM, Spencer GD. Intestinal and hepatic complications of human bone marrow transplantation. Gastroenterology 1986;90:770-784.[Medline]
4. Brodsky R, Topolsky D, Crilley P, Bulova S, Brodsky I. Frequency of veno-occlusive disease of the liver in bone marrow transplantation with a modified busulfan/cyclophosphamide preparative regimen. Am J Clin Oncol 1990;13:221-225.[Medline]
5. Ganem G, Saint Marc Girardin MF, Kuentz M, et al. Venocclusive disease of the liver after allogeneic bone marrow transplantation in man. Int J Radiat Oncol Biol Phys 1988;14:879-884.[Medline]
6. Ozkaynak MF, Weinberg K, Kohn D, Sender L, Parkman R, Lenarsky C. Hepatic veno-occlusive disease post-bone marrow transplantation in children conditioned with busulfan and cyclophosphamide: incidence, risk factors, and clinical outcome. Bone Marrow Transplant 1991;7:467-474.[Medline]
7. Jones RJ, Lee KSK, Beschorner WE, et al. Venooclusive disease of the liver following bone marrow transplantation. Transplantation 1987;44:778-783.[Medline]
8. Ettinger NA, Trulock EP. Pulmonary considerations of organ transplantation. Am Rev Respir Dis 1991;144:213-223.[Medline]
9. Noble PW. The pulmonary complications of bone marrow transplantation in adults. West J Med 1989;150:443-449.[Medline]
10. Hackman RC, Madtes DK, Petersen FB, Clark JG. Pulmonary venooclusive disease following bone marrow transplantation. Transplantation 1989;47:989-992.[Medline]
11. Shulman HM, Gown AM, Wagner DJ. Hepatic venooclusive disease after bone marrow transplantation: immunohistochemical identification of the material within occluded central venules. Am J Pathol 1987;127:549-558.[Abstract]
12. Amento EP, Beck LS. TGF-β and wound healing. In: Bock GR, Marsh J, eds. Clinical applications of TGFβ. West Sussex, England: John Wiley, 1991:115-29.
13. Mustoe TA, Pierce GF, Thomason A, Gramates P, Sporn MB, Deuel TF. Accelerated healing of incisional wounds in rats induced by transforming growth factor-β. Science 1987;237:1333-1336.[Medline]
14. Lynch SE, Colvin RB, Antoniades HN. Growth factors in wound healing: single and synergistic effects on partial thickness porcine skin wounds. J Clin Invest 1989;84:640-646.[Medline]
15. Kelley J, Kovacs EJ, Nicholson K, Fabisiak JP. Transforming growth factor-β production by lung macrophages and fibroblasts. Chest 1991;99:Suppl:85S-86S.[Medline]
16. Hoyt DG, Lazo JS. Alterations in pulmonary mRNA encoding procollagens, fibronectin and

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References

[illegible] Early increases in pulmonary [illegible] transforming growth factor-β in mice sensitive to cyclophosphamide [illegible] fibrosis [illegible]

21. McDonald GB, Shulman HM, Wolford JL, Spencer GD. Liver disease after human marrow transplantation. Semin Liver Dis 1987;7:210-229. [Medline]

22. McDonald GB, Sharma P, Matthews DE, Shulman HM, Thomas ED. Venocclusive disease of the liver after bone marrow transplantation: diagnosis, incidence, and predisposing factors. Hepatology 1984;4:116-122. [Medline]

23. McDonald GB, Shulman HM, Sullivan KM, Spencer GD. Intestinal and hepatic complications of human bone marrow transplantation. Gastroenterology [illegible] [Medline]

24. [illegible] Frequency of veno-occlusive disease of the liver in bone marrow transplantation with a modified busulfan/cyclophosphamide preparative regimen. Am J Clin Oncol [illegible] [Medline]

25. Ganem G, Saint-Marc Girardin MF, Kuentz M, et al. Venocclusive disease of the liver after allogeneic bone marrow transplantation in man. Int J Radiat Oncol Biol Phys 1988;14:879-884. [Medline]

26. [illegible] Hepatic veno-occlusive disease post-bone marrow transplantation in children conditioned with busulfan and cyclophosphamide: incidence, risk factors, and clinical outcome. Bone Marrow Transplant [illegible] [Medline]

27. [illegible] Venocclusive disease of the liver following bone marrow transplantation [illegible] [Medline]

28. [illegible] Pulmonary complications of organ transplantation. Am Rev Respir Dis [illegible] [Abstract]

29. [illegible] Pulmonary complications of bone marrow transplantation in adults. West J Med [illegible] [Medline]

10. [illegible] Clark JG. Pulmonary venocclusive disease following bone marrow transplantation. Transplantation 1989;47:989-992. [Medline]

11. Shulman HM, [illegible] Hepatic venocclusive disease after bone marrow transplantation: immunohistochemical identification of the material within occluded central venules. Am J Pathol 1987;127:549-558. [Abstract]

12. Amento EP, Beck LS. TGF-β and wound healing. In: Bock GR, Marsh J, eds. Clinical applications of TGF-β. West Sussex, England: John Wiley, 1991:115-29.

13. Mustoe TA, Pierce GF, Thomason A, Gramates P, Sporn MB, Deuel TF. Accelerated healing of incisional wounds in rats induced by transforming growth factor-β. Science 1987;237:1333-1336. [Medline]

14. [illegible] Antoniades HN. Role of growth factors in wound healing: single and synergistic effects on partial thickness porcine skin wounds. J Clin Invest 1989;84:640-646. [Medline]

15. Khalil N, Kovacs EJ, [illegible] Transforming growth factor-β production by lung macrophages [illegible] [Medline]

16. Hoyt DG, Lazo JS. Alterations in pulmonary mRNA encoding procollagens, fibronectin and [illegible] [Medline]

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McCormick, L. L., Zhang, Y., Tootell, E., Gilliam, A. C. (1999). Anti-TGF-{beta} Treatment Prevents Skin and Lung Fibrosis in Murine Sclerodermatous Graft-Versus-Host Disease: A Model for Human Scleroderma. *The JI* 163: 5693-5699 [Abstract] [Full Text]

- Coker, R K, Laurent, G J, Jeffery, P K, du Bois, R M, Black, C M, McAnulty, R J (2001). Localisation of transforming growth factor {beta}1 and {beta}3 mRNA transcripts in normal and fibrotic human lung. *Thorax* 56: 549-556 [Abstract] [Full Text]

ZHENG, H., WANG, J., KOTELIANSKY, V. E., GOTWALS, P. J., HAUER-JENSEN, M. (2000). Recombinant Soluble Transforming Growth Factor beta Type II Receptor Ameliorates Radiation Enteropathy in Mice. *Gastroenterology* 119: 1286-1296 [Abstract] [Full Text]

Yong, S.-J., Adlakha, A., Limper, A. H. (2001). Circulating Transforming Growth Factor-{beta}1 : A Potential Marker of Disease Activity During Idiopathic Pulmonary Fibrosis. *Chest* 120: S68-70 [Full Text]

- Anscher, M. S., Marks, L. B., Shafman, T. D., Clough, R., Huang, H., Tisch, A., Munley, M., Herndon, J. E. II, Garst, J., Crawford, J., Jirtle, R. L. (2001). Using Plasma Transforming Growth Factor Beta-1 During Radiotherapy to Select Patients for Dose Escalation. *J Clin Oncol* 19: 3758-3765 [Abstract] [Full Text]

Border, W. A., Noble, N. A. (1994). Transforming Growth Factor {beta} in Tissue Fibrosis. *N Engl J Med* 331: 1286-1292 [Full Text]

MOZES, M. M., BÖTTINGER, E. P., JACOT, T. A., KOPP, J. B. (1999). Renal Expression of Fibrotic Matrix Proteins and of Transforming Growth Factor-{beta} (TGF-{beta}) Isoforms in TGF-{beta} Transgenic Mice. *J Am Soc Nephrol* 10: 271-280 [Abstract] [Full Text]

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- McCormick, L. L., Zhang, Y., Tootell, E., Gilliam, A. C. (1999). **Anti-TGF-**{beta} **Treatment Prevents Skin** and Lung **Fibrosis** in Murine Sclerodermatous Graft-Versus-Host Disease: A Model for Human Scleroderma. *The J Immunol* 163: 5693-5699 [Abstract] [Full Text]
- Coler, R. K., Lamont, G. J., Jeffery, P. K., du Bois, R. M., Black, C. M., McAnulty, R. J. (2000). **Localisation of transforming growth factor** {beta}1 and {beta}3 mRNA transcripts in normal and fibrotic human lung. *Thorax* 55: 549-556 [Abstract] [Full Text]
- ZHENG, H., WANG, J., KOTELIANSKY, V. E., GOTWALS, P. J., HAUER-JENSEN, M. (2000). **Recombinant Soluble Transforming Growth Factor beta Type II Receptor Ameliorates** Radiation Enteropathy in Mice. *Gastroenterology* 119: 1286-1294 [Abstract] [Full Text]
- Yang, S.-L., Adalbja, A. I., Imper, A. R. (2001) Circulating **Transforming Growth Factor** {beta}1 - A Potential **Marker of Disease Activity** During Idiopathic Pulmonary **Fibrosis**. *Chest* 120: 568-70 [Full Text]
- Anscher, M. S., Marks, L. B., Shelburn, T. D., Clough, R., Huang, H., Tisch, A., Munley, M., Herndon, J. E. II, Garst, J., Crawford, J., Jirtle, R. L. (2001). **Using Plasma Transforming Growth Factor Beta**-1 During Radiotherapy to Select Patients for Dose Escalation. *J Clin Oncol* 19: 3758-3765 [Abstract] [Full Text]
- Border, W. A., Noble, N. A. (1994) Transforming Growth Factor {beta} in Tissue **Fibrosis**. *N Engl J Med* 331: 1286-1292 [Full Text]
- MOZES, M. M., BOTTINGER, E. P., JACOT, T. A., KOPP, J. B. (1999). Renal Expression of Fibrotic Matrix Proteins and of Transforming Growth Factor-{beta} (TGF-{beta}) Isoforms in TGF-{beta} Transgenic Mice. *J Am Soc Nephrol* 10: 271-280 [Abstract] [Full Text]

transforming growth factor-β precede bleomycin-induced pulmonary fibrosis in mice. J Pharmacol Exp Ther 1988;246:765-771.[Abstract]
17. Khalil N, Bereznay O, Sporn M, Greenberg AH. Macrophage production of transforming growth factor β and fibroblast collagen synthesis in chronic pulmonary inflammation. J Exp Med 1989;170:727-737.[Abstract]
18. Raghow R, Irish P, Kang AH. Coordinate regulation of transforming growth factor-β gene expression and cell proliferation in hamster lungs undergoing bleomycin-induced pulmonary fibrosis. J Clin Invest 1989;84:1836-1842.[Medline]
19. Phan SH, Gharaee-Kermani M, Wolber F, Ryan US. Stimulation of rat endothelial cell transforming growth factor-β production by bleomycin. J Clin Invest 1991;87:148-154.[Medline]
20. Hoyt DG, Lazo JS. Early increase in pulmonary mRNA encoding procollagens transforming growth factor-β in mice sensitive to cyclophosphamide-induced pulmonary fibrosis. J Pharmacol Exp Ther 1989;249:38-43.[Abstract]
21. Anscher MS, Crocker IR, Jirtle RL. Transforming growth factor-β1 expression in irradiated liver. Radiat Res 1990;122:77-85.[Medline]
22. Czaja MJ, Weiner FR, Flanders KC, et al. In vitro and in vivo association of transforming growth factor-β1 with hepatic fibrosis. J Cell Biol 1989;108:2477-2482.[Abstract]
23. Nakatsukasa H, Nagy P, Evarts RP, Hsia C-C, Marsden E, Thorgeirsson SS. Cellular distribution of transforming growth factor-β1 and procollagen types I, III, and IV transcripts in carbon tetrachloride-induced rat liver fibrosis. J Clin Invest 1990;85:1833-1843.[Medline]
24. Milani S, Herbst H, Schuppan D, Stein H, Surrenti C. Transforming growth factors β1 and β2 are differentially expressed in fibrotic liver disease. Am J Pathol 1991;139:1221-1229.[Abstract]
25. Armendariz-Borunda J, Seyer JM, Kang AH, Raghow R. Regulation of TGFβ gene expression in rat liver intoxicated with carbon tetrachloride. FASEB J 1990;4:215-221.[Abstract]
26. Yamauchi K, Martinet Y, Basset P, Fells GA, Crystal RG. High levels of transforming growth factor-β are present in the epithelial lining fluid of the normal human lower respiratory tract. Am Rev Respir Dis 1988;137:1360-1363.[Medline]
27. Raghu G, Masta S, Meyers D, Narayanan AS. Collagen synthesis by normal and fibrotic human lung fibroblasts and the effect of transforming growth factor-β. Am Rev Respir Dis 1989;140:95-100.[Medline]
28. Castilla A, Prieto J, Fausto N. Transforming growth factors β1 and α in chronic liver disease -- effects of interferon alfa therapy. N Engl J Med 1991;324:933-940.[Abstract]
29. Limper AH, Broekelmann TJ, Colby TV, Malizia G, McDonald JA. Analysis of local mRNA expression for extracellular matrix proteins and growth factors using in situ hybridization in fibroproliferative lung disorders. Chest 1991;99:Suppl:55S-56S.[Medline]
30. Broekelmann TJ, Limper AH, Colby TV, McDonald JA. Transforming growth factor β1 is present at sites of extracellular matrix gene expression in human pulmonary fibrosis. Proc Natl Acad Sci U S A 1991;88:6642-6646.[Abstract]
31. Harrison NK, Argent AC, McAnulty RJ, Black CM, Corrin B, Laurent GJ. Collagen synthesis and degradation by systemic sclerosis lung fibroblasts: responses to transforming growth factor-β. Chest 1991;99:Suppl:71S-72S.[Medline]
32. Varga J, Haustein UF, Creech RH, Dwyer JP, Jimenez SA. Exaggerated radiation-induced fibrosis in patients with systemic sclerosis. JAMA 1991;265:3292-3295.[Medline]
33. Kahaleh MB, LeRoy EC. The immune basis for human fibrotic diseases, especially scleroderma (systemic sclerosis). Clin Aspects Autoimmun 1989;3:19-28.
34. Border WA, Okuda S, Languino LR, Sporn MB, Ruoslahti E. Suppression of experimental glomerulonephritis by antiserum against transforming growth factor β1. Nature 1990;346:371-374.[Medline]
35. Lie TS, Preissinger H, Bach M, et al. The protective effect of cyclosporine against cirrhotic alteration of the liver. Surgery 1991;110:847-853.[Medline]

36. Peters WP, Shpall EJ, Jones RB, et al. High-dose combination alkylating agents with bone marrow support as initial treatment for metastatic breast cancer. J Clin Oncol 1988;6:1368-1376.[Abstract]
37. Proper JA, Bjornson CL, Moses HL. Mouse embryos contain polypeptide growth factor(s) capable of inducing a reversible neoplastic phenotype in nontransformed cells in culture. J Cell Physiol 1982;110:169-174.[Medline]
38. Roberts AB, Lamb LC, Newton DL, Sporn MB, DeLarco JE, Todaro GJ. Transforming growth factors: isolation of polypeptides from virally and chemically transformed cells by acid/ethanol extraction. Proc Natl Acad Sci U S A 1980;77:3494-3498.[Medline]
39. Danielpour D, Dart LL, Flanders KC, Roberts AB, Sporn MB. Immunodetection and quantitation of the two forms of transforming growth factor-β (TGF-β1 and TGF-β2) secreted by cells in culture. J Cell Physiol 1989;138:79-86.[Medline]
40. Freund JE. Modern elementary statistics. 3rd ed. Englewood Cliffs, N.J.: Prentice-Hall, 1967.
41. Bearman SI, Appelbaum FR, Buckner CD, et al. Regimen-related toxicity in patients undergoing bone marrow transplantation. J Clin Oncol 1988;6:1562-1568.[Abstract]
42. Gluckman E, Jolivet I, Scrobohaci M-L, et al. Role of PGE$_1$ to prevent veno-occlusive disease of the liver after bone marrow transplantation. Nouv Rev Fr Hematol 1990;32:133.
43. Piedbois P, Gaspard G, Cordonnier C, et al. Interstitial pneumonitis and veno-occlusive disease of the liver after bone marrow transplantation. Radiother Oncol 1990;18:Suppl 1:125-127.[Medline]
44. Scrobohaci ML, Drouet L, Mogenet-Mansi A, et al. Liver veno-occlusive disease after bone marrow transplantation: changes in coagulation parameters and endothelial markers. Thromb Res 1991;63:509-519.[Medline]
45. Weiner RS, Horowitz MM, Gale RP, et al. Risk factors for interstitial pneumonia following bone marrow transplantation for severe aplastic anaemia. Br J Haematol 1989;71:535-543.[Medline]
46. Wingard JR, Mellits ED, Jones RJ, et al. Association of hepatic veno-occlusive disease with interstitial pneumonitis in bone marrow transplant recipients. Bone Marrow Transplant 1989;4:685-689.[Medline]
47. Lucas C, Baldi LN, Fendly BM, et al. The autocrine production of transforming growth factor-β1 during lymphocyte activation: a study with a monoclonal antibody-based ELISA. J Immunol 1990;145:1415-1422.[Abstract]
48. Massague J. The transforming growth factor-β family. Annu Rev Cell Biol 1990;6:597-641.
49. Kovacina KS, Steele-Perkins G, Purchio AF, et al. Interactions of recombinant and platelet transforming growth factor-β1 precursor with the insulin-like growth factor II/mannose 6-phosphate receptor. Biochem Biophys Res Commun 1989;160:393-403.[Medline]
50. Jirtle RL, Carr BI, Scott CD. Modulation of insulin-like growth factor-II/ mannose 6-phosphate receptors and transforming growth factor-β1 during liver regeneration. J Biol Chem 1991;266:22444-22450. [Erratum, J Biol Chem 1991;266:24860.][Abstract]
51. Jirtle RL, Meyer BA. Liver tumor promotion: effect of phenobarbital on EGF and protein kinase C signal transduction and transforming growth factor-β1 expression. Dig Dis Sci 1991;36:659-668.[Medline]

37. Hooper JA, Bjornson CL, Moses HL. Mouse embryos contain polypeptide growth factor(s) capable of inducing a reversible neoplastic phenotype in nontransformed cells in culture. J Cell Physiol 1982;112:[illegible].

38. Perkins AB, Lamb LC, Newton DL, Sporn MB. Polypeptide growth factor(s) from virally transformed cells: isolation of polypeptides from viral and chemically transformed cells by acid/ethanol extraction. Proc Natl Acad Sci U S A 1982;[illegible].

39. Danielpour D, Dart LL, Flanders KC, Roberts AB, Sporn MB. Immunodetection and quantitation of the two forms of transforming growth factor-β (TGF-β1 and TGF-β2) secreted by cells in culture. J [illegible] 1989;[illegible].

40. Proud JK, [illegible]. Biochem Biophys Res Commun 1988;[illegible].

41. Beauman SR, Anderson CD, Pittelkow MR, Derynck R. Keratinocytes express a unique transforming growth factor-α. Mol Cell Biol 1988;8:[illegible].

42. Quintanilla I, Jolivet J, Sundsmo M-L, et al. Role of PDGF in pre-eclamptic vaso-occlusive disease. [illegible]

43. [illegible]

44. [illegible]

45. [illegible]

46. [illegible]

47. [illegible]

48. [illegible]

49. [illegible]

50. [illegible]

51. [illegible]